

Claim 4. The method according to claim 1, wherein said plant cells or tissue comprise a root culture.

**R E M A R K S**

Favorable reconsideration of the subject application is respectfully requested in view of the amendments above and the comments below.

Claims 1-20 are pending in the present application. Claims 13 and 14 are canceled herein. Accordingly, claims 1-12 and 15-20 are presented for examination on the merits.

Claim 1 has been amended to incorporate the limitations of claims 13 and 14, which have been canceled and to make that which was implicit in the claim language explicit. Claims 3 and 4 have been amended to correct clerical errors. Claim 3 has also been amended to delete reference to "algal suspension". Accordingly, no new issues are raised and no new matter is added by these amendments to the claims.

It is respectfully submitted that the amendments to the claims place the subject application in condition for allowance, or at the very least, limit the issues for appeal. As such, it is respectfully submitted that the amendments should be entered at this time.

**I. Rejection of Claims 1-20 Under 35 U.S.C. § 112, First Paragraph**

Claims 1-20 are rejected under 35 U.S.C. § 112, first paragraph as allegedly being inadequately enabled by the specification. The Examiner states that

transformation of monocots mediated by Agrobacterium would have required undue experimentation at the time of the invention (Sept. 1999).

This rejection is respectfully traversed as follows.

The Examiner argues that the scope of the present claims is not enabled by the specification because transformation of monocots by Agrobacteria was not routine at the time of the invention. The Examiner also states that Basczynski et al. represents the first teaching of such transformation. However, Basczynski et al. is directed to stable transformation of monocots and teaches a method of stably transforming monocots using an Agrobacterium and a site-specific recombinase that ensures the insertion of the recombinant polynucleotide into the plant genome. However, this reference is not the first disclosure of Agrobacterium mediated transformation of monocots.

Contrary to the Examiner's assertion, transformation of monocotyledenous plants by use of Agrobacteria and the transient expression of recombinant genes had been demonstrated prior to the present invention. (See J. Bacterol. (1987), 169(4):1745-6; Narasimhulu et al. (1996), Plant Cell, 8(5):873-86; and Grimsley et al., (1986), Proc. Natl. Acad. Sci. USA, 83:3282-6, copies enclosed). The prior art discloses that transient expression of monocots had been achieved using methods similar to those used to transform dicots.

The Examiner has also pointed out prior art that discloses problems associated with the stable transformation of monocots. However, such literature is not dispositive of a lack of enablement of the claimed invention. The prior art cited by the Examiner

addresses issues of stable transformation of monocots and does not address transient transformation and expression in monocots.

In contrast to the prior art cited by the Examiner, the present invention is directed to methods of transiently transforming plant cells and/or tissue (both monocots and dicots) on a large scale in liquid media. Applicants have discovered that it is not necessary to establish stable transformation of plants in order to produce sufficient quantities of recombinant polypeptide. Applicants have established a large scale plant biomass production method that can be manipulated to increase transient recombinant gene expression.

The prior art demonstrates that transient transformation of both monocots and dicots mediated by Agrobacteria was obtainable at the time of the invention. Therefore, it is not necessary that the specification include an example of that which the prior art has amply demonstrated. The present invention is not simply the transformation of monocots by Agrobacteria, and is not directed to the stable transformation of monocots. Instead, the present invention provides a method which can be applied to the large scale production of recombinant protein in plants that eliminates the need to establish stable transformation and stably transformed cell lines. The inventive method also advantageously eliminates the need to culture plant cells under conditions suitable for regeneration of plants or plant tissues.

The specification provides an example of such a transiently transformed plant cell culture and the isolation of recombinant polypeptide therefrom. Thus, the specification provides an enabling disclosure of the full scope of the claimed process.

Accordingly, the rejection of claims 1-20 under 35 U.S.C. § 112, first paragraph is respectfully traversed.

## **II. Rejection of Claims 1-18 Under 35 U.S.C. § 103(a)**

Claims 1-18 are rejected under 35 U.S.C. § 103(a) as being unpatentably obvious over Goodman et al. The Examiner states that the cited prior art reference teaches production of polypeptides in plants and asserts that the sequence of steps in such method is an obvious design choice.

This rejection is respectfully traversed as follows.

Goodman et al. merely teaches transformation of plants using Agrobacteria as vector to stably transform plants or leaf discs, which are then grown under conditions to produce transformed plants. Goodman et al. does not teach co-culturing of plant cells/tissue with Agrobacteria, followed by liquid culturing of the co-culture, nor is there any disclosure or suggestion that recombinant polypeptide is obtainable from such co-culture. More particularly, Goodman et al. does not teach bioreactor co-culture of plant cells and/or plant tissue.

Furthermore, this reference neither discloses, nor suggests the method of the present invention in which plant cells or tissue are grown in liquid culture or grown and introduced to bioreactor culture conditions, infected with Agrobacteria and then grown in liquid culture under conditions favoring transient expression of recombinant polypeptide. In particular, Goodman et al. does not teach monitoring conditions during the a liquid culturing process in order to determine optimal conditions for plant cell growth, timing of inoculation, or optimal conditions for gene transfer and transient expression of the

recombinant polypeptide. Goodman et al. simply does not teach or suggest the conditions for cell growth or inoculation that are set forth in the claims and does not teach or suggest use of an auxotrophic Agrobacteria to control bacterial cell growth during production as set forth in the dependent claims. More particularly, Goodman et al. does not teach bioreactor culturing or liquid culturing of transformed plant cells. As such, the cited reference does not render the present application obvious.

Accordingly, the rejection of claims 1-18 under 35 U.S.C. § 103(a) over Goodman et al. is respectfully traversed.

### **III. Rejection of Claims 1, 13, 19 and 20 Under 35 U.S.C. § 103(a)**

Claims 1, 13, 19 and 20 are rejected under 35 U.S.C. § 103(a) as being unpatentably obvious over Goodman et al. in view of Baszczynski et al. The Examiner states that it is an inherent property of the claimed invention that the vector is stably incorporated into the plant genome and also asserts that transient expression is an inherent feature of Agrobacterial transformation of plant cells. Thus, the Examiner asserts that Goodman et al. teaches transient transformation of plant cells. The Examiner concludes, therefore, that the combined prior art teaches all of the limitations of the claimed invention.

Applicants respectfully disagree with the Examiner's conclusion.

The present invention is directed to a method of transiently transforming plant cells for large scale production of recombinant polypeptide. In the present invention plant cells are grown in liquid media, inoculated with Agrobacteria and grown under conditions that enable transient expression of recombinant polypeptide in liquid culture.

The culture is monitored to determine optimal growth conditions and timing for inoculation and harvesting of the recombinant polypeptide.

In contrast, Goodman et al. and Baszczynski et al. both teach the necessity for transferring transformed plant cells from liquid culture to a medium suitable for growth of a callus and other plant tissues. These references do not teach or suggest liquid cultures of transformed plant cells for the production of recombinant polypeptide.

It is irrelevant that transient expression may occur (although this has not been demonstrated) during the processes disclosed by these two prior art references. It is also irrelevant that some stable transformation may occur during the claimed process (although this has not been demonstrated) for the simple reason that the prior art processes and the present invention are directed to very different plant transformation, culturing and harvesting processes. For example, Baszczynski teaches a method of plant transformation that utilizes viruses having a circular genome or replication intermediate (col. 5, line 60) and a recombinase in order to create a modified viral replicon.

There is nothing in either of the cited prior art references that suggests liquid culture of Agrobacteria-transformed plant cells, much less on a large scale production. In fact, these references teach away from liquid culturing of transformed plant cells. As such, the cited combination of prior art fails to render the present invention obvious.

Accordingly, the rejection of claims 1, 13, 19 and 20 under 35 U.S.C. § 103(a) over the cited prior art is respectfully traversed.

It is respectfully submitted that the present application, as amended above, is in condition for allowance, an early notification thereof being earnestly solicited.

Please charge any shortage in fees due in connection with the filing of this paper, including extension of time fees, to deposit Account 500417 and please credit any excess fees to such account.

Respectfully submitted,

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**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

*Please cancel claims 13 and 14, without prejudice.*

*Please amend claims 1, 3, and 4 as follows:*

Claim 1 (Amended). A method for [recombinantly and] transiently [producing a]  
transforming plant cells or plant tissue for the large scale production of recombinant  
polypeptide [in a plant tissue,] comprising:

- i) providing a plant tissue sample to a bioreactor or cultivating plant cells or  
plant tissue in liquid medium in a bioreactor[;] under conditions suitable for growth of the  
cells or tissue,
- ii) [adding a sample of Agrobacterium] inoculating the plant cells or plant  
tissue with a culture of Agrobacteria when suitable growth of the cells or tissues is  
obtained, the Agrobacteria containing a vector comprising a nucleotide sequence  
encoding the recombinant polypeptide [to the plant tissue sample;],
- iii) [mixing the plant tissue sample with] culturing the plant cells or plant tissue  
and the Agrobacterium [so that the nucleotide sequence is transferred to the plant;]  
under conditions suitable for transfer of the nucleotide sequence to the plant cells or the  
plant tissue to thereby produce transiently transformed plant cells or plant tissue,
- iv) [allowing the plant tissue] growing the transiently transformed plant cells or  
plant tissue in liquid medium under conditions that enable the transiently transformed  
plant cells or tissue to transiently express the recombinant polypeptide; and
- v) [separating the polypeptide from the mixture] isolating the recombinant  
polypeptide from the transiently transformed cells or tissue,

wherein the conditions are monitored during step (I), (iii), and/or (iv) by measuring optical density, pH, temperature, nutrient levels, oxygen, conductivity, refractive index, osmolarity, calcium level of the medium, protein expression level, or a combination thereof.

Claim 3 (Amended). The method according to claim 1, wherein said plant tissue [sample] is a plant cell [or algal cell] suspension culture.

Claim 4 (Amended). The method according to claim 1, wherein said plant cells or tissue [sample is] comprise a root culture.

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